

Attorney Docket No. P64029US0

Serial No. 09/423,622

**REMARKS**

Claims 48-61, presented hereby, are pending.

Claims 1-47 have been cancelled, without prejudice or disclaimer.

Present independent claim 48 combines subject matter of claims 36 and 38. Present claim 49 contains subject matter from claim 38. Present claims 50-54 correspond to claims 39, 42-44, and 46, respectively, made dependent on present claim 48. Claim 55 is an independent claim, which represents subject matter of present claim 48 styled as a "method of treating a lesion of neuronal tissue." Present claims 56-61 correspond to present claims 49-54, but are dependent on claim 55.

Applicants wish to thank the Examiner for the indication of allowable subject matter (Office Action page 8), i.e., the suggested claim reading:

A method of enhancing axonal regeneration comprising locally administering an inhibitor substance that inhibits basal membrane formation of lesioned postcommisural fornix to enhance axonal regeneration, and wherein the inhibitor substance is an anti-collagen IV antibody or  $\alpha,\alpha'$ -dipyridyl (DPY).

The present claims incorporate language from the aforesaid allowable claim in order to resolve issues raised in the rejection under §112, second paragraph.

Claims 36-38 and 42-47 were rejected under 35 U.S.C. §112, first paragraph, for allegedly lacking enablement and under 35 U.S.C. §112, second paragraph, allegedly being indefinite. Reconsideration is requested with respect to the rejections under §112, first paragraph, and under §112, second paragraph, in view of the changes in the claims, effected hereby, and the following remarks.

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The limitation of claim 36 by incorporation of the subject matter of claim 38 is intended to resolve the rejection against the breadth of claim 36. Since, now, specific classes of compounds are recited, there is no undue burden for identifying compounds that fall within the claim scope.

The rejection against the term "improvement of the CNS" under item 9 of the Office Action (page 7) is addressed by the language in the preamble of the present claims. Of course, it was never meant to claim an improvement of the CNS after a lesion but, rather, a regeneration of neuronal tissue and, consequently, the recovery of CNS functionality that had been lost due to the neuronal lesion.

The presently claimed invention teaches the use of compounds that inhibit formation of basal lamina in order to treat the neuronal damage - and accompanying loss of CNS functioning - caused by lesions in the nervous system, e.g., the severing of a nerve.

Attached hereto (Appendix, pages i-v) is an experimental report showing the extraordinary recovery of nerve-damaged rats following treatment in accordance with the presently claimed invention. The fornix was damaged in one series of test rats, and in another series the spinal cord was damaged. In these experimental paradigms two different, but defined, fiber tracts were transected (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Figs. 1 and 2). These experiments comprised both compartments of the central nervous system, i.e., the brain and the spinal cord, and focused on the axonal regeneration of two different cell types, (i.e. fornix: limbic system fiber tract, non-motor tract; spinal cord: corticospinal tract, motor tract) to show the universality of response (i.e., axonal regeneration) with respect to *cell type* in the treatment of CNS

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lesions, as presently claimed, with basal-lamina-formation inhibitors (using prolyl 4-hydroxylase inhibitors as examples). In *each case* (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Fig. 3) basal-lamina formation was *effectively inhibited* by local application of different iron chelators (fornix: 2,2'-bipyridine; spinal cord: 2,2'-bipyridine-5,5'-dicarboxylic acid and desferrioxamine) and anti-collagen type IV antibodies (fornix). The different agents used were, in fact, selected in order to determine the universality, with respect to neuronal-cell type, of effective treatment in accordance with invention presently claimed, i.e., by using prolyl 4-hydroxylase inhibitors to prevent basal lamina formation in damaged nerve tissue. For both brain-lesion and spinal-cord-lesion paradigms, administration of basal-lamina-formation inhibitors (i.e., prolyl 4-hydroxylase inhibitors) resulted in axonal regeneration (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Figs. 4 and 5) and associated recovery of CNS functioning. The recovery of functioning by regeneration in the fornix lesion is shown, necessarily, by electrophysiological improvement (i.e., because there is no behavioral outcome measurable in fornix lesioned animals); whereas, the recovery of CNS functioning accompanying regeneration spinal lesion is demonstrated, directly, by locomotion testing (open field test, walking analysis performed by cat-walk testing) and by testing fine-motor movements based on sensory motor coupling (testing by walking on a horizontal ladder) (fornix lesion: see drawings in WO 98/51708; spinal lesions, Appendix, Figs. 6-8).

Accordingly, the instant specification provides sufficient teaching to enable the skilled person to practice the invention as presently claimed, i.e., how to administer the substances that inhibit the

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formation of the basal membrane and, so, effect neuronal regeneration with a corresponding recovery of CNS functioning.

The protocol described in the present specification for the brain-lesion model (i.e., the transected postcommissural fornix) is readily extrapolated to treatment of spinal-cord damage. This is demonstrated by behavioral testing (Appendix), which could be used because of the suitability of the lesion paradigm, wherein local application of, even, prolyl 4-hydroxylase inhibitors other than 2,2'-bipyridine (i.e., the inhibitors 2,2'-bipyridine-5,5'-dicarboxylic acid and desferrioxamine [deferrioxamine]) leads to the same positive result (i.e., with regard to prevention of basal lamina formation and the promotion of axonal regeneration and functional recovery) as observed in connection with the brain-lesion model (discussed above).

With these two experimental setups the applicability of treatment in accordance with the presently claimed invention is shown for CNS lesions, in general. In each lesion paradigm, and for each inhibitor substance used, a dose-response test (which is the normal procedure for pharmacological testing) was necessary to determine the useful concentration of the inhibitor substance to be administered to prevent basal lamina formation. Therefore, applicants submit that the described protocol enables the skilled person to practice the presently claimed invention, e.g., reproducing the experiments performed and results obtained for the brain and spinal cord lesion paradigms.

Thus, the requirements of enablement under §112, ¶1, are satisfied in connection with the subject matter presently claimed.











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### Medical Dictionary

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axon hillock

Go

Main Entry: ax-on

Pronunciation: 'ak-'san

Variant(s): also ax-one /-'sɒn/

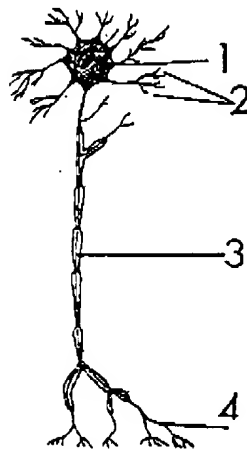
Function: noun

: a usually long and single nerve-cell process that usually conducts impulses away from the cell body

-ax-on-al /'ak-sən-'sɒl; 'ak-'sæn-, -'sɒn-/ adjective

[axon illustration]

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neuron: 1 cell body, 2 dendrite, 3 axon, 4 nerve ending

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EXHIBIT A

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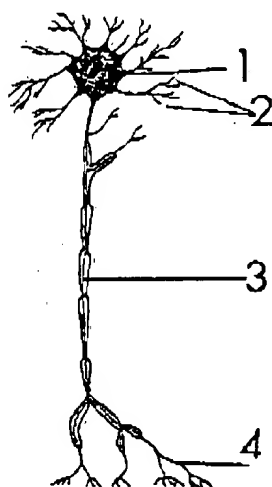
Function: noun

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-ax-o-nal /'ak-sän-'äl; 'ak-'sän-, -'sön-/ adjective

[axon illustration]

<http://www2.merriam-webster.com/mw/arg/neuron.htm>



neuron: 1 cell body, 2 dendrite, 3 axon, 4 nerve ending

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**EXHIBIT A**

Experiments in the Molecular Neurobiology Laboratory, Dept. of Neurology, Heinrich-Heine-University Duesseldorf, of Prof. Müller have shown that local application of iron chelators (different from 2,2'-bipyridine [syn.:  $\alpha,\alpha'$ -dipyridyl], namely a 5,5'-dicarboxylic acid derivative of 2,2'-bipyridine (BPY-DCA) and another iron chelator, namely deferoxamine) prevents basal lamina formation following spinal cord lesions thus enabling axonal regeneration and functional recovery of locomotor and fine motor movements in the adult rat.

In spinal lesions (Fig. 1, Fig. 2A) a basal lamina forms (Fig. 2A) as it has been shown in the deep brain lesion of the transected postcommissural fornix (Fig. 2B)

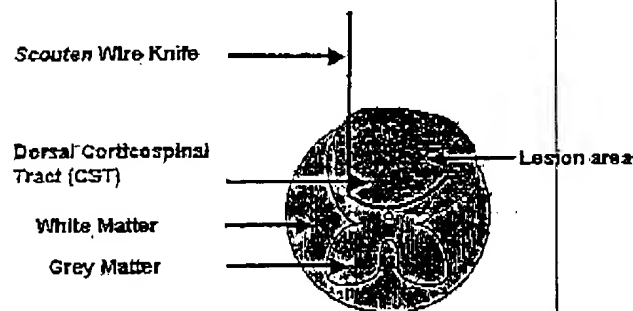


Fig. 1: Lesion of the dorsal part of the spinal cord with a scouten wire knife

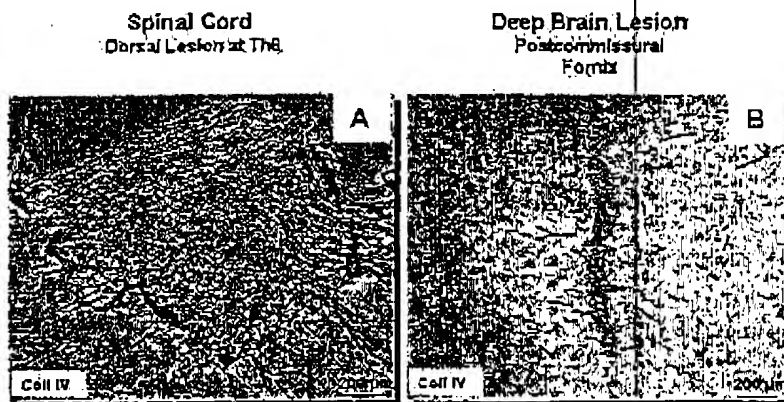


Fig. 2: Basal lamina formation visualized by immunohistochemical staining for Col type IV in a dorsal spinal cord lesion (A) and the lesioned postcommissural fornix (B).

To show the universality of different prolyl 4-hydroxylase inhibitors we injected a 5,5'-dicarboxylic acid derivative of 2,2'-bipyridine (BPY/DCA) and another iron chelator, deferoxamine (syn: desferrioxamine, Fig. 3). The use of different prolyl 4-hydroxylase inhibitors states in our opinion the universal applicability of these substance classes. Dosage has to be established individually for each inhibitor.



receiving the prolyl 4-hydroxylase inhibitor show a overall performance like sham operated animals 15 weeks post operation. All treated animals show coordinate walking pattern.

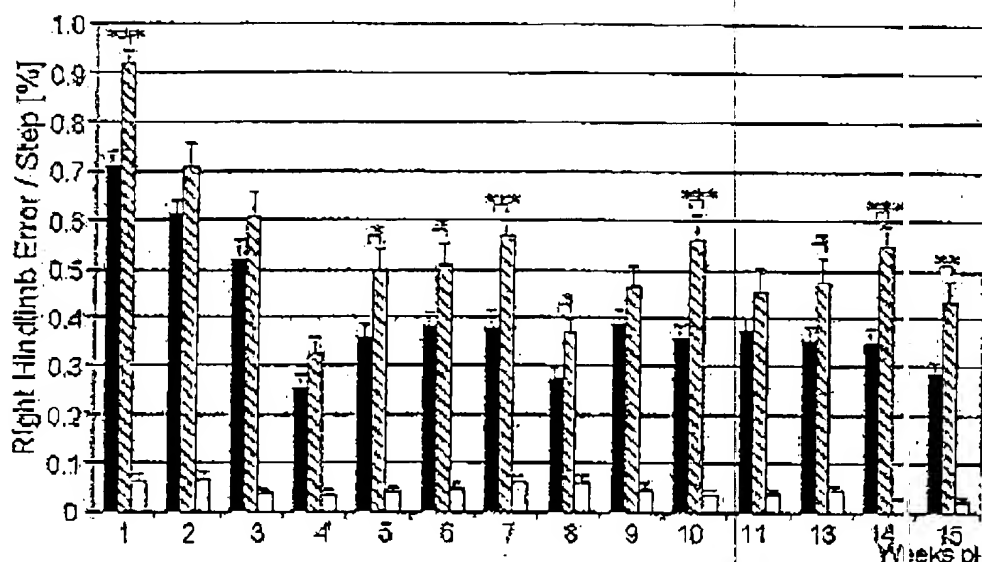


Fig. 7: Horizontal ladder test to evaluate fine motor movements. Filled (black) bars represent animals treated with prolyl 4-hydroxylase inhibitor, striped bars represent buffer control animals and blank bars represent sham operated animals. Treated animals perform significantly better (less footfalls) compared to controls.

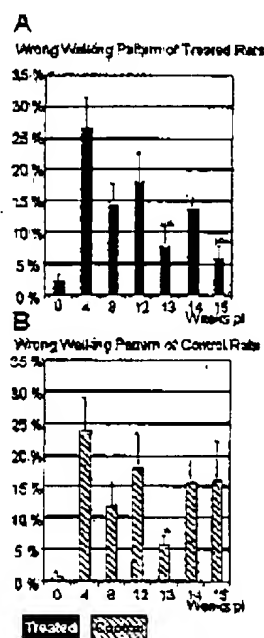


Fig. 8: (A) and (B) show results of the analysis of animals over ground locomotion on the CatWalk device. The mean percentage of wrong walking patterns  $\pm$  standard error of the mean before and after operation is shown for treated animals in (A) and for control animals in (B). Significant differences of data pl week 8 - 15 in comparison to pl week 4 are depicted by

#### APPENDIX

asterisks (\*  $p < 0.05$ , \*\*  $p < 0.005$ , \*\*\*  $p < 0.001$ ). Treated animals show significant improvement of the regular walking pattern on several testing days, but control animals remain at a high level of irregular walking patterns, except for week 13. Additionally significant differences could be observed between pre-lesion baseline values and p week 15 and 16 for control animals ( $p < 0.05$ ), where treated animals show no significant difference between performance results of pre-lesion and p1 week 15 and 16 performances, indicating a recovery to pre-lesion performance.

#### Reference List

1. Basso DM, Beattie MS, Bresnahan JC (1995) A sensitive and reliable locomotor rating scale for open field testing in rats. *J Neurotrauma* 12: 1-21.
2. Hamers FP, Lankhorst AJ, van Laar TJ, Veldhuis WB, Gispens WH (2001) Automated quantitative gait analysis during overground locomotion in the rat: its application to spinal cord contusion and transection injuries. *J Neurotrauma* 18: 187-201.
3. Metz GA, Merkler D, Dietz V, Schwab ME, Fouad K (2000) Efficient testing of motor function in spinal cord injured rats. *Brain Res* 883: 165-177.

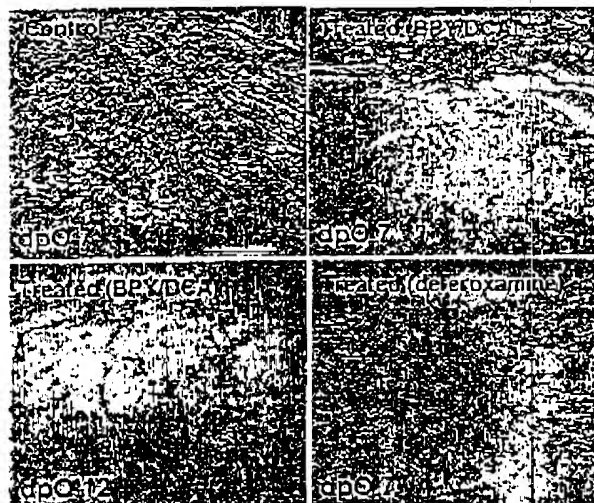


Fig. 3: Prevention of basal lamina formation in spinal cord lesions by immediate injection of different prolyl 4-hydroxylase inhibitors at 7 and 12 days post operation (dp0). Basal lamina formation visualized by immunohistochemical staining for Coll type IV. Magnification bar in control picture for all: 500  $\mu$ m.

Prevention of basal lamina formation in the traumatically injured spinal cord resulted in axonal regeneration across the lesion site (Fig. 4) and long distance axonal elongation of regenerated fibers in the denervated distal spinal cord (Fig. 5). To visualize a specific fiber population (dorsal corticospinal tract, CST) a tracer substance (biotinylated dextran amine, BDA) was injected into the sensory motor cortex of the animals. This tracer substance is transported in fibers that have a connection to the cell bodies, i.e. if CST fibers in or beyond the lesion site carry the tracer these fibers are regenerated.

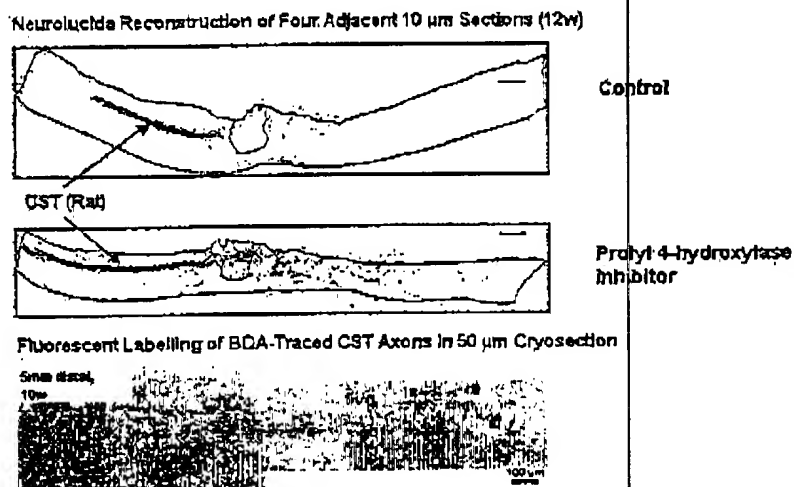


Fig. 4: Drawings: Neurolucida reconstructions of four adjacent parasagittal 10  $\mu$ m sections, CST traced with BDA, 12 weeks post operation. In control animals transected, BDA-traced CST fibers do not enter the lesion area and do not elongate in the denervated distal part of the spinal cord. In animals treated with a prolyl 4-hydroxylase inhibitor transected, BDA-traced CST fibers cross the lesion site and elongate in the distal part of the spinal cord. Magnification bars: 1mm. Photomicrograph: Fluorescent labelling of regenerating BDA-traced CST fibers 5 mm distal to the lesion site, 10 weeks post operation.

## APPENDIX

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